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PRINCIPAL INVESTIGATOR: Muriel T. Davisson, Ph.D.

CONTRACTING ORGANIZATION: Jackson Laboratory
Bar Harbor, Maine 04609-1500

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Davisson, M.T.

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13. ABSTRACT *(Maximum 200 words)*

The overall goal of this project is to develop and maintain a resource of mouse models for breast cancer research. Forty-five strains carrying induced mutations have been identified and accepted for importation into The Jackson Laboratory (TJL) Induced Mutant Resource (IMR) repository for breast cancer research models. The importation process frees mice of infectious pathogens. Embryos or gametes are cryopreserved. Correct nomenclature is assigned, efficient breeding strategies are developed, and genotyping protocols are modified for optimal efficiency and accuracy. Strain availability is announced in several media, including a page on the IMR's World Wide Web site, accessed through TJL's WWW home page.

A principal aim of this project is to transfer relevant mutations to a defined genetic background. Nine mutations are being transferred, including 7 to the FVB/NJ inbred background. Reports by others and our own observations suggest that tumor characteristics may be altered as a consequence of background strain modifiers. Congenic strains require characterization of tumor onset and type.

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FOREWORD

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MJD In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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(NA) In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Muriel T. Davisson 11/11/98
Pt - Signature / Date

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INTRODUCTION

Mouse mutants that provide models for human breast cancer and model systems to study the function of genes implicated in breast cancer are being produced in large numbers in many research laboratories, world-wide. Genetically engineered models are powerful tools for a) determining gene function in normal mammary gland development; b) understanding mechanisms of mammary carcinogenesis; and c) testing new therapies.

The purpose of the repository for breast cancer-related mutants at The Jackson Laboratory (TJL) is to make genetically defined mice of assured health status available to the world research community.

The specific aims of this project are to:

1. Select mutants with importance to breast cancer research for importation into the IMR. Selection involves:
   A) Identifying relevant strains
   B) Determining criteria for selection
   C) Encouraging participation by investigators holding transgenic and targeted mutants
   D) Addressing legal considerations
   E) Cooperating to avoid duplication of efforts

2. Import (by hysterectomy rederivation) transgenic and targeted mutant mice with importance for breast cancer research into defined health status breeding rooms at The Jackson Laboratory;

3. Maintain and expand breeding colonies of imported strains for cryopreservation, strain development, and distribution;

4. Develop accurate and rapid methods for typing stocks for inclusion of transgenes or targeted mutations;

5. Develop improved mouse models for breast cancer research by transferring mutant genes to selected inbred backgrounds conferring specific experimental advantages;

6. Distribute mutant and control mice to scientific investigators on a cost recovery basis;

7. Maintain data on imported mutants and subsequently developed new strains in a computerized database for maintenance of nomenclature, information on mutants held in the resource, and tracking information of mice.

BODY OF THE REPORT

SPECIFIC AIM 1: SELECT MUTANTS WITH IMPORTANCE TO BREAST CANCER RESEARCH FOR IMPORTATION INTO THE IMR.

A. Identifying Relevant Strains

Drs. Sharp and Tennent are responsible for identifying relevant strains for the breast cancer repository. Table 1 lists the strains accepted to the repository in the 04 year. Several of the strains accepted this year carry mutations in key cell cycle regulatory genes, which have been implicated in human breast cancer. The effects of these mutations on mammary gland morphology has not been systematically investigated. By including them in the repository,we can bring them to the attention of breast cancer researchers who may not be aware of them.

Table 1. Strains accepted for importation to the transgenic repository for breast cancer research in 03 year.

Strain Designation	Type of mutation	JR #	Ref	Distribution Status
129/Sv-*Rbl1*^{tm1Tyj}	KO	JR2988	[1]	Accepted - not yet available
129/Sv-*Rbl1*^{tm1Tyj}*Rbl2*^{tm1Tyj}	Double KO	JR3241	[2]	Accepted - not yet available
129/Sv-*Rbl2*^{tm1Tyj}	KO	JR2989	[2]	Accepted - not yet available
C57BL/6-*Bax*^{tm1Sjk}	KO	JR 2994	[3]	Accepted, not yet available
C57BL/6J-TgN(WapTAg)1Knw	transgenic	JR 3135	[4]	Minimal distribution
C57BL/6J-TgN(WapTAg)3Knw	transgenic	JR 3189	[4]	Minimal distribution
129/Sv-*Prlr*^{tm1Cnp}	transgenic	JR 3141	[5]	Accepted - not yet available
C57BL/6-*Prlr*^{tm1Cnp}	transgenic	JR 3142	[5]	Accepted - not yet available
C57BL/6-*Cdh3*^{tm1Hyn}	KO	JR 3180	[6]	Accepted - not yet available
STOCK TgN(Trp53 A135V)#Ber	transgenic	JR 3262	[7]	Accepted - not yet available**
129/Sv-Bcl2^{tm1Mpin}	KO	JR 3082	[8]	Accepted - not yet available

Brief descriptions of strains accepted (from literature cited in Table 1, personal communications with original investigators, and other sources as cited)

129/Sv-*Rbl1*^{tm1Tyj}

The *Rb* family members are important cell cycle regulatory genes, with overlapping but distinct effects on cell cycle progression. This strain carries a null mutation in the retinoblastoma-like 1 gene, which encodes the p107 protein that has limited homology to the Rb1 product. RB family members participate in the cyclin/cyclin dependent kinase complex-mediated control of G_1 cell cycle progression, and are regulators of transcriptional activation of E2F family members and other transcription factors necessary for cell cycle progression. In human breast cancer cells, synthetic progestin-mediated growth inhibition is preceded by reduction in both pRB and p107 phosphorylation [9], suggesting that p107 may be important in the progestin signaling pathway in mammary cells. Additionally, p107 has been implicated in DNA damage-induced growth arrest in cultured cells.

Mice deficient in p107 are viable and fertile, with no increased morbidity or mortality up to 24 months of age observed in mutant mice with a mixed C57Bl/6 and 129 background. Mutants generated on an inbred 129/Sv background did show subtle thikening of the forlimb bones. The mammary glands were not examined, but no deficiency in lactation was reported.

p107-deficient mice may be useful for examining the role of Rb family members in steroid-mediated proliferation and differentiation, as well as in DNA damage-induced growth arrest. Combined mutation stocks can be generated by crosses with mice carrying transgenes or targeted mutations affecting other Rb

family members and cyclins, some of which are also available through the repository. p107-deficient mice can also be mated to transgenics carrying specific oncogenes targeted to the mammary gland to examine the function of p107 in mammary carcinogenesis.

129/Sv-*Rbl2*^{tm1Tyj}

Rbl2 is another member of the retinoblastoma-like gene family that encodes the p130 protein. Overexpression of p130, like p107, arrests cells in the G_1 phase of the cell cycle, and p130 also associates with E2F transcription factor family members. In human breast cancer cells, p130 is phosphorylated in response to estrogen stimulation, indicating it is part of the estrogen signal transduction pathway [10].

Mice deficient in p130 are viable and fertile and display no abnormalities at birth. Mammary glands were not examined. Mice of this strain may be useful for determining the involvement of p130 in controlling proliferation and differentiation in the mammary gland. In combination with strains carrying altered expression of other *Rb* family members or cyclin/cyclin dependent kinases, these mice can be used to examine signal transduction pathways.

129/Sv-*Rbl1*^{tm1Tyj}*Rbl2*^{tm1Tyj}

This strain carries both the *Rbl1* and *Rbl2* null mutations described above. In contrast to single-mutant mice, mice deficient in both Rbl family members die at or shortly after birth. Abnormalities are first evident between embryonic days 15.0 and 16.0. Embryos were smaller than normal littermates and had reduced bone deposition in the ribs and long bones of the limbs. Bone abnormalities may be caused by loss of p107 and p130-mediated control of chondrocyte proliferation.

These mice are useful sources of embryonic cells and fetal tissues in which the overlapping but distinct functions of Rb family members may be determined.

C57BL/6-*Bax*^{tm1Sjk}
129/Sv-Bcl2^{tm1Mpin}

Bcl2-associated X protein, encoded by *Bax*, forms heterodimers with and antagonizes the repression of apoptosis mediated by BCL2. The ratio of BAX to BCL2 expression is critical to occurrence of an apoptotic response to various stimuli. When BCL2 is in excess, homodimers for that protein are formed and repress cell death. When BAX\alpha is in excess, BAX homodimers are formed and cell death occurs. In the involuting mammary gland of mice, programmed cell death of individual alveolar cells is correlated with increased expression of *Bax* [11]. The role of alterations in *BCL2* family members' control of apoptosis in human breast cancer is not well understood and is under investigation. In human breast cancer cells, levels of *BAX* expression are inversely correlated with *TRP53* expression [12]. Bax expression was also correlated with response to chemotherapy in women with metastatic breast cancer [13]. Loss of both *BAX* and *BCL2* expression, which indicates severe disregulation of the apoptotic pathway, may be prognostic in node-negative breast carcinomas[14], although another study has shown that *BCL2* but not *BAX* expression was correlated with high tumor grade, proliferative rate, and apoptosis [15]. The use of targeted mutant mice to determine the function of these apoptosis-regulatory proteins in normal and neoplastic mammary tissue should help resolve their roles in human breast cancer.

Bax-deficient mice are viable, but display lineage-specific alterations in apoptotic regulation. Thymocytes and B cells are hyperplastic, and the ovaries are characterized by unusual, atretic follicles in which excess granulosa cells are found. Mammary glands were not examined. Males are infertile with testes characterized by disordered seminiferous tubules with accumulated atypical premeiotic germ cells.

Bcl2 deficient mice are viable, but are reduced in size compared to wild-type littermates. There is substantial death of *Bcl2* -/- mice at 7 to 8 weeks of age, but mutants may survive at least one year. Mutants are characterized by loss of CD8+ and CD4+ lymphocytes, with little loss of B cells. Mutant mice also have defects in non-hematopoietic tissues, including polycystic kidney disease, small auricles, and hypopigmentation of the hair. Although the paper reports no defects were found in "glandular epithelium", no studies of mammary gland morphogenesis or function are described.

These mice are useful for further delineating the function of *Bcl2* and *Bax* in the normal involution of the mammary gland. They may be mated to mice carrying transgenic oncogenes predisposing to mammary carcinoma in order to examine the role of *Bcle* family members in mammary carcinogenesis and metastasis.

C57BL/6J-TgN(WapTAg)1Knw
C57BL/6J-TgN(WapTAg)3Knw

Simian virus 40 T antigen (SV40Tag) is a multifunctional regulatory protein that stimulates gene transcription and forms complexes with cell cycle-regulatory proteins such as TRP53 and RB1 that are implicated in human breast cancer. SV40Tag expression was targeted to the mammary epithelium of lactating C57BL/6J female mice using the whey acidic protein (WAP) promoter. The inbred background of these transgenic mice is useful for studies on genetic and immunological parameters of carcinogenesis.

Multiparous female mice of the WapTag1 lineage develop mammary adenocarcinomas with an average latency of 13 months. The histopathological phenotype is heterogeneous; papillary, ductal, tubular, and sometimes solid phenotypes are observed. Those tumors with a papillary morphology closely resemble human papillary carcinomas. Tumors arise adjacent to morphologically normal mammary epithelium or hyperplastic lesions. Mammary carcinomas are extremely rare in virgin female mice. Male mice, and those female mice that do not develop mammary carcinoma develop undifferentiated soft tissue sarcomas. Multiparous females of the WapTag3 lineage have hyperplastic mammary glands and occasionally develop mammary adenocarcinomas by 6 months of age. At this age, both males and females of this lineage develop osteosarcomas arising from the *os petrosum* and renal adenocarcinomas.

129/Sv-*Prlr*^{tm1Cnp}
C57BL/6-*Prlr*^{tm1Cnp}

Prlr encodes the prolactin receptor, which is also the receptor for other lactogenic hormones. These hormones are critical for the development of the mammary gland during pregnancy and lactation.

Female mice deficient in *Prlr* are sterile due to abberant estrous cycles, abnormal preimplantation development of eggs, or no implantation of blastocysts. They do not exhibit pseudopregnancy. Males show slightly delayed fertility. Mammary development is markedly affected. Homozygotes have no mammary development and do not lactate. Heterozygotes are unable to lactate after the first pregnancies, but attain some degree of lactation as they age or after multiple pregnancies. Serum prolactin levels are increased 60 - 100 fold in both males and females. Maternal behavior is diminished in primiparous animals. Bone development is reduced in homozygote mutants.

C57BL/6-*Cdh3*^{tm1Hyn}
Cdh3 encodes P-cadherin, a member of the cadherin family of glycoproteins involved in cell-cell adhesion. P-cadherin appears to be important in maintaining the structural integrity of epithelial tissues. In the mouse mammary gland, P-cadherin is normally expressed in the cap cells of the end bud and in the myoepithelial cells, both cell types that are important in mammary ductal branching morphogenesis. In the human, P-cadherin is expressed in the myoepithelial cells. Studies of cadherin in human breast cancer have focused primarily on E-cadherin expression in the tumor tissue, although cadherin function in the surrounding myoepithelium has not been determined.

Mice deficient in P-cadherin are viable and fertile, and females are able to lactate. Virgin P-cadherin-null females have precocious differentiation of the mammary gland, such that alveolar-like buds resemble those in a female in early stages of pregnancy. With age, glands become hyperplastic and dysplastic, with abnormal lymphocyte infiltration. These mice are useful for examining mechanisms of myoepithelial control of mammary epithelial growth, and of precancerous lesions in the mammary gland.

STOCK TgN(Trp53 A135V)#Ber
Mutations in the *Trp53* tumor suppressor are found in a wide variety of human tumors, including breast cancers. Many of these are point mutations that occur in the highly conserved region in the middle third of the gene. These mutations may encode a mutant protein that behaves in a dominant-negative fashion. The involvement of interactions of mutant and wild-type proteins in loss of cell-cycle control is an important area of cancer research.

This strain carries a null mutation in *Trp53* and is transgenic for a point mutation resulting in an Arg-Val substitution at the 175th amino acid. Homozygous mice are viable and fertile, but show a high incidence of tumors, particularly lung adenocarcinomas, osteosarcomas, and lymphomas. Mammary tumors were infrequent. Tumor latency is accelerated in comparison to *Trp53* -/- mice.

These mice may be useful in complementation studies with mice transgenic for mammary oncogenes to examine the contribution of a dominant negative form of Trp53 on tumor progression.

B. Criteria for Selection

Drs. Sharp and Tennent present identified strains to the Genetic Resources Committee, chaired by Dr. Davisson, for a decision regarding selection. Criteria for selection of mutants is based on existing guidelines for importing mice to the Laboratory's Genetic Resources. These are: 1) the immediate need for use in biomedical research; 2) the numbers of requests for mice being received by the investigators who created them; 3) the potential for future research; 4) the time and effort needed to replace or recreate the mutant; and 5) the uniqueness of the mutation. We continue to accept strains to be cryopreserved directly, without maintaining a breeding colony for distribution of live mice. These are strains that carry a scientifically valuable mutation of interest to a small number of laboratories, for which we do not expect a large demand. Orders for mice are filled by recovering animals from cryopreserved gametes or embryos. Institutions that are equipped to recover mice from cryopreservation may request shipment of frozen embryos or gametes. We expect that this method of distribution will increase, as more institutions establish cryopreservation laboratories. Mice recovered from cryopreservation have effectively gone through a rederivation process and can be introduced into high level barrier facilities at institutions where rederivation is required.

C. Encouraging Participation by Investigators Holding Transgenic Mice

Efforts to encourage participation in the Breast Cancer Resource have been very successful this year. Distribution figures shown under progress reported in Specific Aim 6 show a doubling of mice distributed in the current year. Knowledge about the Resource has grown such that all of the strains submitted this year were offered by investigators, in contrast to previous years when we actively sought strains to achieve the requisite 10 strains per year for importation. Methods for encouraging participation this year both for the Breast Cancer Resource in particular and the Induced Mutant Resource in general are listed below.

1) An IMR presentation is given at all courses and workshops held at The Jackson Laboratory. These courses include the *Short Course in Medical and Experimental Mammalian Genetics*, given annually in association with the Johns Hopkins University School of Medicine; *Experimental Genetics of the Laboratory Mouse*, a graduate and post-graduate level course led by an international faculty; the *Cryopreservation* course, and special workshops and conferences focused on animal models. In October, 1997, a meeting on "The Mouse in Mammary Carcinogenesis Research" was held at TJL for about 70

participants, funded in part by a supplement to DAMD17-94-J-4016 (see section on use of supplemental funds below). This meeting included a session on insuring community access to mutant strains of mice, a poster describing the Breast Cancer Resource, as well as sessions introducing participants to online sources of information about mutant mice.

2) IMR personnel accept all relevant speaking and writing opportunities to disseminate information and invite participation in the program. These include:

- Genetically Engineered Mouse Models of Human Disease. *Probing White Matter Disorders.* The United Leukodystrophy Foundation. DeKalb, IL, July 1997.
- Overview of the Induced Mutant Resource and Genetically Engineered Mouse Models. The Science and Technology Commission of The Peoples Republic of China, Bar Harbor ME, September 1997.
- Overview of the Induced Mutant Resource and Genetically Engineered Mouse Models. Otsuka Pharmaceuticals, Bar Harbor ME, September 1997.
- Future Directions of The Induced Mutant Resource at The Jackson Laboratory. Glaxo Wellcome, Bar Harbor ME, October 1997.
- Mouse Models of Human Disease - Genetically Engineered Mice. Robert Wood Johnson Research Foundation, Johnson & Johnson Pharmaceuticals, Raritan NJ, October 1997.
- Mouse Models for Heart Disease - The Induced Mutant Resource at The Jackson Laboratory. *Genetic Approaches In Complex Heart, Lung and Blood Diseases.* The Jackson Laboratory, Bar Harbor ME, October 1997.

Publications about the Induced Mutant Resource include:

- Tennent BJ, Sharp JJ, Washburn LL, Schweitzer P, Sundberg JP, Silva KA, Davisson MT. 1997. Reporitory of Induced mutant Mice for Breast Cancer Research. Proceedings of Breast Cancer Research Program, USAMRMC.
- Sharp JJ. 1998. Genetically Engineered Mouse Models of Human Disease. Proceedings of the Fourth Symposium on Probing White Matter Disorders, Molecular and Chemical Neuropathology.
- Davisson MT, Sharp JJ. 1998. Repositories of Mouse Mutations and Inbred, Congenic and Recombinant Inbred Strains, In: Systematic Approach to Evaluation of Mouse Mutations, Sundberg JP and Boggess D (eds.), CRC Press, (in press).
- Sharp JJ, Linder CC, Mobraaten LE. 1998. Genetically Engineered Mice - The Induced Mutant Resource at The Jackson Laboratory. In: Methods in Enzymology (in press).

3) A separate site has been created for the breast cancer repository on The Jackson Laboratory website (http://www.jax.org/). Mutant lists are available, linked to information on the gene (through links with *Mouse Genome Database*), strain background, availability, phenotype, references, and ordering information. Genetic typing protocols for some strains are posted on the WWW as well. The site also has a table summarizing characteristics of the preclinical models within the resource, including data on tumor incidence and latency, sex and breeding status of susceptible mice, metastatic incidence and pathology. This table was developed in response to a request from the breast cancer Preclinical Models working group at the National Cancer Institute.

4) The breast cancer resource site is linked to the Biology of the Mammary Gland homepage maintained by Dr. Lothar Hennighausen (http://alice.dcrt.nih.gov/~mammary/). This Website offers written descriptions and scanned images of histomorphology from mice carrying induced or spontaneous mutant mutations that affect mammary gland development and mammary cancer.

5) IMR personnel and Dr. Carol Linder, Technical Services Advisor for TJL, distribute information about the IMR program in general and the breast cancer repository in particular at selected scientific meetings where The Laboratory trade booth is exhibited. In the 04 year of this grant, the booth has been exhibited at 4 meetings: Neuroscience - October 26 - 29, 1997 - New Orleans; American Heart Association - November

9 - 12, 1997 - Orlando; Amer. Assoc. for Cancer Research - March 28 - April 1, 1998 - New Orleans; Experimental Biology/Immunology - April 19 - 21, 1998 - San Francisco. A new edition of the Breast Cancer catalog was prepared and over 150 copies were distributed at the AACR meeting alone. The exhibit booth is a valuable forum for exchanging information about new mutants presented at these meetings and for disseminating information about mutants already in the breast cancer repository.

D. Addressing Legal Considerations

The legal negotiations at The Jackson Laboratory are the responsibility of David Einhorn, Esq., The Jackson Laboratory in-house counsel. In the 04 year of this grant, a negotiation with DuPont over the breadth and scope of the "Oncomouse" patent was finally completed. Thirty-one strains within the breast cancer resource that are more likely to develop neoplasms as a result of an inserted gene fall under DuPont's definition of an "Oncomouse" in the agreement. Under the terms of this agreement, a letter is sent to all investigators requesting mice considered "Oncomice" to notify them that their institution must obtain a license from DuPont. The Jackson Laboratory submits a quarterly list of purchasers of "Oncomice" to DuPont, but shipment of mice is not dependent on confirmation of licensure. A small committee of IMR personnel meets to determine which strains must be considered "oncomice" under DuPont's definition as set forth in the agreement. Criteria used are peer-reviewed data clearly showing a statistically significant increase in neoplasias in genetically-engineered mice compared to appropriate controls. When this agreement with DuPont was reached, Drs. Sharp and Tennent sent a letter to each investigator who had submitted a strain now considered an "oncomouse" strain, explaining the agreement and expressing our regret that we were obligated to so designate the strain. Investigators offering new strains that are considered "oncomice" are informed of this designation at the time of acceptance.

Some of the strains within the resource are now covered by multiple patents and some require licensing agreements from the originating institution as well as a party such as DuPont that has a patent purporting to cover a class of mice. Progeny of two newly established crosses (discussed under Specific Aim 5 below), will require commercial companies to obtain licensing from two originating institutions and from DuPont. Academic purchasers will need to obtain a license from DuPont only. We are deeply concerned about the proliferation of licensing requirements and commercialization of basic research tools, such as mice, that may impede the distribution of mice to investigators to address critical issues in in the scientific search to understand breast cancer.

E. Cooperating to Avoid Duplication of Efforts

We have found that duplication of effort is best avoided by contact with the investigator holding the mice requested. Investigators who initiate the contact usually have offered their mutants only to The Jackson Laboratory. To screen for potential duplication, a form is sent to each potential provider of mutant animals asking if the animal is being offered to other institutions. Investigators are also asked for their knowledge of any similar animal being produced elsewhere. Investigators holding mice relevant for breast cancer research are very helpful in suggesting mice for the repository and discussing the specific experimental advantages of similar models.

SPECIFIC AIM 2. IMPORT (BY HYSTERECTOMY REDERIVATION) TRANSGENIC AND TARGETED MUTANT MICE WITH IMPORTANCE FOR BREAST CANCER RESEARCH INTO DEFINED HEALTH STATUS BREEDING ROOMS AT THE JACKSON LABORATORY

Importation is completed for 32 of the 34 of the strains accepted in the 01-03 years. The FVB/N - TgN(WapMyc)212Bri strain will be scheduled for importation now that legal negotiations with DuPont are complete. The CD1-TgN(MtTGFA)42Lmb strain was reimported after the first set of rederived progeny developed mega-esophagus before breeding colonies could be established. No fertile females were recovered from the second set of rederived progeny. This strain was maintained by the original investigator as homozygotes on the CD1 genetic background. The Jackson Laboratory does not distribute wild type CD1 mice, so there was no opportunity to rescue the mutation by crossing to a CD1 +/+ female. The

remaining males were outcrossed to the closely related Swiss strain, SWR/J. The original investigator has previously found that the phenotype of tumor susceptbility changed markedly in F1 progeny of a CD1-TgN(MtTGFA)42Lmb X FVB/N cross. Consequently, offspring of the SWR cross will be monitored for tumor onset and the types of tumors will be determined by histological analysis. This strain may be reimported on the CD1 background if obvious deviations in phenotype are found. The FVB-TgN(MMTVCCND1) strain has not yet been scheduled for importation due to ongoing licensing negotions.

The status of strains accepted in the 04 year is shown in Table 2. Two strains are being distributed. The *Rbl1* and *Rbl2* null mutations were received as the double mutant 129/Sv-*Rbl1*^{tm1Tyj}*Rbl2*^{tm1Tyj} strain and can be separated by breeding upon demand. Four additional strains are in the importation process; progeny from most have been recovered but vigorous breeding colonies are not yet established. Two strains have been requested but the original investigator has not yet sent mice. An additional strain accepted in June, 1998 is scheduled for importation when isolator space is available.

Table 2. Importation of strains accepted in the 04 year

Strain	Status
129/Sv-*Rbl1*^{tm1Tyj}	importation
129/Sv-*Rbl1*^{tm1Tyj}*Rbl2*^{tm1Tyj}	importation
129/Sv-*Rbl2*^{tm1Tyj}	importation
C57BL/6-*Bax*^{tm1Sjk}	importation
C57BL/6J-TgN(WapTAg)1Knw	minimal distribution
C57BL/6J-TgN(WapTAg)3Knw	minimal distribution
129/Sv-*Prlr*^{tm1Cnp}	requested, not yet received
C57BL/6-*Prlr*^{tm1Cnp}	requested, not yet received
C57BL/6-*Cdh3*^{tm1Hyn}	importation
STOCK TgN(Trp53 A135V)#Ber	scheduled for importation
129/Sv-Bcl2^{tm1Mpin}	in importation

SPECIFIC AIM 3. MAINTAIN AND EXPAND BREEDING COLONIES OF IMPORTED STRAINS FOR CRYOPRESERVATION, STRAIN DEVELOPMENT AND DISTRIBUTION

Breeding colonies are established for 29 of the 32 strains imported in the 01-03 years. Two strains are maintained solely as frozen embryos, and one strain has been discontinued following transfer of the transgene to different genetic backgrounds that were more economical to maintain.

Seventeen of the strains accepted in the 01-03 years have been or are being cryopreserved. Since many of the strains are difficult to establish in breeding colonies, we have used most of the available mice from strains in demand to fill requests from investigators before cryopreserving them.

Mutations that arrive on a nonstandard genetic background may be transferred to an inbred strain background by repeated backcross. At present 9 mutations are being transferred to other backgrounds conveying specific experimental advantages. Strain development is discussed more fully under Specific Aim 5.

Most of the strains in the breast cancer repository must be maintained as heterozygotes or hemizygotes. In several strains transgenic females do not lactate, requiring that the strain be maintained either by breeding carrier males to inbred, wild-type females, or by fostering litters, or both. When strains are maintained

using hemizygous breeders, all offspring must be genotyped to identify carriers. Blood samples are obtained primarily by retro-orbital sampling, and DNA extracted and typed as described in Specific Aim 4 below.

SPECIFIC AIM 4. DEVELOP ACCURATE AND RAPID METHODS FOR TYPING STOCKS FOR INCLUSION OF TRANSGENES OR TARGETED MUTATIONS

The IMR allele typing program is the responsibility of Dr. Sharp. Virtually all mouse strains in the IMR require genetic typing to confirm the presence of the transgene in transgenic strains, or the genotype of targeted mutants. Genetic typing is required to identify carrier animals (heterozygotes) for strains being backcrossed onto a defined genetic background (in excess of 90% of all strains), to identify hemizygous animals for those transgenic strains supplied as hemizygotes, and to identify heterozygotes for those strains where the homozygous mutants are embryonic lethals or do not reproduce. Allele typings are carried out using the polymerase chain reaction (PCR) because it is rapid, the reaction conditions may be standardized and it does not require the use of radioisotopes. The numbers of mice genotyped annually continues to increase substantially each year. Strains that must be distributed as young mice, or strains characterized by very early onset tumors, are now typed on an accelerated schedule so that they can be released for distribution or set up in mating rapidly.

Allele typing protocols for IMR strains are first developed in the IMR Development Laboratory under the supervision of Peter Schweitzer, Ph.D. Dr. Schweitzer is responsible for developing and testing all genetic typing protocols for each new strain and is also responsible for overseeing the correct use of these protocols. He has contacted all of the researchers supplying mice to the breast cancer repository and is optimizing protocols for the strains already received. He reviews all typing results and provides these protocols to researchers requiring assistance. The protocols are regularly posted on the World Wide Web.

The Jackson Laboratory has a pending application at NIH for an ABI PRISM 7700 Sequence Detection System, which has received a favorable priority score. The IMR typing program is planning to use this equipment, if funded, to replace the time-consuming quantitative Southern blotting that they now use to confirm homozygosity in transgenic strains.

SPECIFIC AIM 5. DEVELOP IMPROVED MOUSE MODELS FOR BREAST CANCER RESEARCH BY TRANSFERRING MUTANT GENES TO SELECTED INBRED BACKGROUNDS CONFERRING SPECIFIC EXPERIMENTAL ADVANTAGES

Because of the marked strain differences in susceptibility to spontaneous, hormonally-induced, and chemically induced mammary adenocarcinomas and the number of as yet unidentified "background" modifying genes (including MMTV proviral insertions) participating in susceptibility, it is essential to place transgenes and targeted mutant genes on defined, inbred backgrounds. Appropriate selection of mutant alleles and inbred backgrounds will increase the utility of these models for breast cancer research. Seven mutations (Table 3) are being transferred to the FVB/NJ background because of the common use of this strain to make transgenic mice, low incidence of spontaneous mammary tumors, and lack of milk-transmitted MMTV or replication-competent endogenous MMTV provirus. The transgene is also maintained on the background on which it was originally received.It was our intention to expand matings at the fifth generation of backcrossing, identify carrier females and examine them for tumor latency and histotype. With the termination of funding from DAMD17-94-J-4016, this characterization can only take place if sufficient alternative funding can be obtained.

Table 3. Strains backcrossed to FVB/NJ strain background

Original strain	Generation
B6,129-*Ccnd*^{tmi}	N9
B6D2-TgN (MMTVTGFA)254Rjc	N7
B6D2-TgN (MMTVTGFA)29Rjc	stopped produceing, N6
B6,129-*Trp53*^{tm1Tyj}	N7
B6, 129-*Rb1*^{Tm1Tyj}	N9
B6D2 TgN(MMTVTGFB1)46Hlm	N9
SJL-TgN(Wnt1)1Hev	N8

Wnt1

Preliminary observations on tumor latency in successive backcross generations of SJL-TgN(Wnt1)Hev mice to FVB/NJ suggested that strain background may influence tumorigenesis in this model. Animals were inspected during weekly cage changing for visually observable tumors. Mice were necropsied by TJL's Pathology Service when mice were judged "sick", as determined by tumor size, behavior, or scruffy pelt. At the N5 generation, the colony was expanded and progeny have been set aside to observe tumor incidence and latency. Mice have accumulated slowly into the study, due to consistent demand for this strain. Outside requests always take precedence over filling experimental groups within the resource. Four group-housed virgin females in the observed group have developed mammary tumors with lifespans ranging from 69-206 days. Mice are killed when tumors are visually observed. Additional virgin and a cohort of multiparous females continue to be observed.

Because of the very poor reproductive performance of SJL-TgN(Wnt1)1Hev mice, the transgene was "rescued" by mating carrier males to B6SJLF1 female mice. This strategy was chosen because the strain was originally prepared on a B6SJL hybrid background. There was a marked improvement in reproduction, and this stock now accounts for the majority of *Wnt1* transgenic mice distributed. Mice are being held for determination of tumor latency and frequency. To date, four group-housed virgin females have developed mammary tumors with lifespans ranging from 50 to 221 days. The large variation in tumor latency will require that additional, substantial groups be observed to discover whether there are background effects on this phenotype.

Trp53

Cooperativity with strain-characteristic susceptibility alleles may also be established by transferring mutant genes to different inbred backgrounds with defined susceptibility to mammary carcinogenesis. Previous progress reports have discussed the phenotypes observed in intermediate backcross animals during the transfer of *Trp53* and *Rb1* null mutations to the BALB/cJ and C3H/HeOuJ backgrounds. The BALB/cJ strain was chosen because spontaneous papillary adenocarcinomas with histomorphology closely resembling the human infiltrating ductal carcinoma have been observed in the TJL Animal Resource colony of this strain. *Trp53*-deficient BALB/cJ mice will also be useful to investigators using chemical or MMTV-induced mammary tumors. N5 mice are being distributed, and backcrossing is continuing to N10, when further characterization of tumor types and latencies will be completed. In contrast to all other strains carrying the Trp53 null mutation, the BALB/cJ strain can not be maintained by mating homozygous males to heterozygous females because the males do not breed and become moribund very early. The C3H/HeOuJ strain was chosen because mice develop a high frequency of MMTV-induced mammary adenocarcinomas and are widely used in breast cancer research. The cause for the pronounced decrease in mammary tumorigenesis detected in the Animal Resource colony of this strain has not yet been resolved. We have fixed the backcross lines generated to transfer the *Trp53* and *Rb1* at N5 and are distributing these strains.

We have also examined a small number of FVB-*Trp53*^{tm1Tyj} homozygous mice at N5. Tumors found to date have been hemangiosarcomas or poorly differentiated angiosarcomas.

Combined transgenic strains

In the 04 year, two crosses were made to provide progeny that carry complementary transgenes affecting mammary carcinogenesis. The phenotypes of progeny from both crosses have been published previously, as noted below.

JR3181: FVB-TgN(MMTVneu)202MulTgN(Trp53R172H)8512Jmr Mammary tumors are first observed after two rounds of pregnancy and lactation at 112 days; the median latency was 154 days. In a cohort examined [16], all mice developed mammary tumors before 200 days of age. The neoplasms are of high grade and growth fraction as indicated by variable nuclear and cytological morphology, anaplasia, aneuploidy, and higher rates of mitosis and apoptosis. Metastatic incidence was not reported, although metastases are sometimes observed (personal communication with the originators). We are holding mice to obtain metastatic incidence data in our colony.

JR3182: FVB-TgN(MMTVneu)202MulTgN(MMTVTGFA)29Rjc In bitransgenic virgin mice, the mammary trees of both bitransgenic and MMTV-TGF-a mice show extensive lobuloalveolar development comparable to a normal FVB lactating mouse [17]. The alveoli of the bitransgenic mice had a denser cell lining in the walls. Their mammary glands were characterized by epithelial hyperplasia and dysplasia with stromal inflammation. In virgin mice, mammary tumors first arose at about 100 days; the median latency was approximately 180 days. Tumors were multifocal and involved the entire mammary epithelium. Metastatic incidence was not reported, and we are holding mice to obtain data on metastases in our colony.

SPECIFIC AIM 6. DISTRIBUTE MUTANT AND CONTROL MICE TO SCIENTIFIC INVESTIGATORS ON A COST RECOVERY BASIS

The scientific community is made aware of the availability of strains through the informational program described in Specific Aim 1 C. In total, 27 strains are now being distributed from the Breast Cancer Repository, some of which are different inbred strains carrying the same mutation. More than 2500 mice have been distributed to more than 300 investigators. Many of these are breeding pairs to establish new colonies. These figures show significant growth since the 03 year in which more than 1000 mice were distributed to 130 investigators. Only nine of the strains distributed had orders totaling more than 50 mice, however, demonstrating the importance of a resource that makes both the high demand and low demand strains available to accommodate diverse research needs.

We distribute breeding pairs, mutant, and control mice on a "first-come, first-served" basis, a system that is employed successfully by other resource colonies at TJL. When we receive a request for a large number of mice from one strain, we ship those mice in small groups interspersed with shipments to other researchers; this ensures the equitable distribution of our resources.

Many strains in low demand are maintained in small breeding colonies, which cannot provide large numbers of mice for any single order. We recognize that delays in availability and a lack of communication have been problematic for some investigators requesting mice. TJL has developed a strategic plan to provide more accurate information on the availability of mice from these colonies, and to reduce delays in providing mice. New, self-explanatory levels of availability have been established and all stocks will be assigned to one of these based on biological parameters and cost-effectiveness: 7 days, unlimited availability; 30 days, limited availability; weaning to order; and frozen embryos only. TJL is investing in a new computer system to monitor inventory and improve communication between colony and customer-service personnel. The customer service staff, long recognized for their knowledge about mice, is being expanded to accommodate the increased interest in induced mutant mice. TJL also has a technical support staff that is being expanded to 4 people including an expert in transgenic and targeted mutant mice.

SPECIFIC AIM 7. MAINTAIN DATA ON IMPORTED MUTANTS AND SUBSEQUENTLY DEVELOPED NEW STOCKS IN A COMPUTERIZED DATABASE FOR MAINTENANCE OF NOMENCLATURE, INFORMATION ON MUTANTS HELD IN THE RESOURCE AND TRACKING INFORMATION OF MICE

The IMR database is maintained by Phyllis Mobraaten, Information Specialist, to track internal management information on all IMR strains. The database allows multiple-user access with both JAX-only and public views. The IMR database contains strain information such as gene description, phenotype, husbandry, typing methods, and nomenclature. All the genetic databases at The Jackson Laboratory use correct nomenclature following the guidelines of the International Committee on Standardized Genetic Nomenclature for Mice. When strains are accepted for importation to the breast cancer repository, appropriate nomenclature is agreed upon with the original investigator, approved by the Mouse Genome Database Nomenclature Coordinator, and a Laboratory Registration Code is obtained from the International Central registry maintained by the Institute for Laboratory Animal Resources (ILAR, NRC, NAS). Strain information from the IMR database is routinely posted on the World Wide Web as described in Specific Aim 1C.

Information maintained in this database also is used to retrieve statistical data. The development of both the public and private components of this database will continue for the foreseeable future. Other currently independent departmental databases at TJL contain information that is complementary with that in the IMR database. We have designed and implemented an interface named "strain tracker" for tracking information between the Importation and the IMR databases. Work is in progress to provide a similar system to enable the IMR database to "interact" with the Production department's computer system for colony and customer order tracking.

USE OF SUPPLEMENTAL FUNDS

A supplement to this award was obtained in the 04 year for the support of the meeting, "The Mouse in Mammary Carcinogenesis Research", Bar Harbor, ME, October 6-8, 1997. The conference was organized by Drs. Lothar Hennighausen (NIDDK, NIH), Susan Ross (University of Pennsylvania) and Barbara Tennent (The Jackson Laboratory). More than 70 participants attended the meeting, in which leaders in the preparation and analysis of mouse models of mammary cancer as well as young investigators presented their results. Invited speakers are listed in Table 4. Additional sessions included a hands-on comparative pathology workshop, a demonstration of available databases and other web-based resources, and a poster session.

Table 4. Faculty of The Mouse in Mammary Carcinogenesis Research

Robert Cardiff, M.D., Ph.D., University of California, Davis
Lewis Chodosh, M.D., Ph.D. University of Pennsylvania School of Medicine,
Gerald R. Cunha, Ph.D. University of California, San Francisco
Chuxia Deng, Ph.D. LBM, NIDDK, NIH
Wafik El-Deiry, Howard Hughes Medical Institute, University of Pennsylvania
Jai Evans, LBM, NIDDK, NIH
Priscilla Furth, University of Maryland
Marina.Glukhova, INSTITUT CURIE - Research Division
Lothar Hennighausen, Ph.D. LBM, NIDDK, National Institutes of Health
Nelson Horseman, University of Cincinnati
Marc Lippman, Lombardi Cancer Center
Dr. Leif R. Lund, Finsen Laboratory
William J. Muller, Ph.D. McMaster University
Alexander Mikitin, University of Texas, San Antonio
Christopher Ormandy, Garvan Institute of Medical Research
Thomas Reid, NHGRI, NIH
Jeffrey Rosen, Ph.D. Baylor College of Medicine
Susan Ross, Ph.D., University of Pennsylvania
Barbara Tennent, Ph.D. The Jackson Laboratory
Dr. Sefton Wellings, M.D.,Ph.D, Emeritus, University of California, Davis
Zena Werb, Ph.D., University of California, San Francisco
Anthony Wynshaw-Boris, Ph.D., M.D. NHGRI, NIH

The meeting was evaluated using a voluntary questionnaire, of which 20 were returned. Response was very positive and emphasized that the focus on mouse mammary gland biology and carcinogenesis provided a basis for in-depth discussion. The small sessions led by Drs. Cardiff and Wellings on evaluating tumor histopathology and comparative mouse/human pathology of the mammary gland were consistently cited as among the most valuable. Specific suggestions for improvement included several requests for expanded sessions on histopathology and hands-on training components demonstrating techniques for mammary gland transplantation and culture, and analytical techniques. These elements are included in a workshop on Techniques for Modeling Mammary Cancer in Mice planned for October, 1999 at The Jackson Laboratory. The workshop will be offered in conjunction with a conference on Modeling Human Mammary Cancer in Mice, which builds on the success of the initial meeting funded with support from DAMD17-94-J-4016.

CONCLUSIONS

This year, ten induced mutant strains of particular relevance to breast cancer research have been identified and accepted for importation into The Jackson Laboratory Induced Mutant Resource repository for breast cancer research models. Correct nomenclature has been assigned to each strain, an important step for disseminating information about mutant strains and reducing duplication of effort. Efficient breeding strategies for each strain have been developed, and typing protocols have been obtained and are being modified for optimal efficiency and accuracy. The availability of these strains is being announced in several media, including a dedicated site accessed through The Jackson Laboratory's WWW home page. These pages are linked to other sites that convey genetic and phenotypic information.

Many of the strains accepted to the repository have impaired reproductive performance as a consequence of the transgene or strain background. We have implemented ovary transfer and ovary cryopreservation to speed colony establishment and preservation of strains. We have initiated outcrosses where necessary to preserve the mutation.

Many induced mutant stocks of particular relevance to breast cancer research have been created and maintained on mixed genetic backgrounds, which limits their usefulness for most genetic studies. At present, many of these models for breast cancer are transgenic stocks carrying oncogenes or growth factors with expression directed to the mammary gland. We are transferring mutant genes to the FVB/NJ background by repeated backcross, while monitoring for any changes in phenotype.

We continue our outreach to the breast cancer research community. The first in a series of meetings on mouse models for breast cancer was held at The Jackson Laboratory this fall, which allowed us to showcase the repository. We have collaborated with the NCI working group on preclinical models for breast cancer to make information about available models more easily accessible. The mouse promises to be a vital tool for functional genetics research on the normal and diseased mammary gland. Interest in genetically engineered models for mammary carcinogenesis continues to grow, and several targeted mutants are now available to examine gene function in the developing mammary gland. There is clearly a continuing need for the Breast Cancer Repository.

List of Meeting Abstracts and Publications Supported by DAMD17-94-J-4016

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List of Personnel Supported by DAMD17-94-J-4016

BOSOM, KENNETH E	**SENIOR BIOMEDICAL TECHNOL**
BROWN, AARON C	**SENIOR BIOMEDICAL TECHNOL**
BURGETT, BRADFORD	**SENIOR BIOMEDICAL TECHNOL**
DAVISSON, MURIEL T	**SR STF SCIENTIST/DIR OF GEN RS**
L'ITALIEN, LAWRENCE E	**SR BIOMEDICAL TECHNOL**
SCHWEITZER, PETER	**RESEARCH ASSOCIATE**
SCOTT, VALERIE E	**PROFESSIONAL BIOMED TECH**
SEAVEY, KATHLEEN M	**SENIOR LABORATORY ASST II**
SHARP, JOHN J	**RESEARCH SCIENTIST/SUPER IMR**
SILVA, KATHLEEN A	**BIOMEDICAL TECHNOLOGIST**
SMITH, RICHARD S	**RESEARCH SCIENTIST**
TENNENT, BARBARA J	**COORDINATOR, BREAST CA RESOURCE**
WASHBURN, LINDA L	**SEN PROFESSIONAL ASSISTANT**

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